

Long DNA fragments (0.1-1 Mb) are required in many polymer physics studies, especially implementing single-molecule approaches. Previously we presented a membrane reactor designed to produce samples of sub-megabase DNA fragments. This reactor is capable of extracting and purifying high quality genomic DNA and additionally perform various reactions such as restriction enzyme digestion, intercalation with fluorescent dyes, and labeling with sequence-specific tags. This 125 μ l volume reactor performs preparations significantly faster than routine procedures and is completely automated.

To extend the ability of the reactor to work with smaller bacterial loads (10^6 cells vs. 10^8 cells), we recently introduced an axisymmetric flow focusing mode. In this mode, flow fields created within the chamber focus the bacterial cells to a small area in the center of the membrane. This arrangement helps to limit the interaction of deformable DNA coils with membrane nanopores which leads to decreased sample losses. It also enables elution of the sample in a smaller volume and at 5-10-fold higher concentration. Implementation of flow splitting during the elution process helps to further increase the DNA concentration by an order of magnitude.

This work focuses on experimental and numerical characterization of the flow fields employed in the reactor. Experimental study was performed with 160 kb DNA and 240 kDa proteins. Numerically, the semidilute DNA solution on the membrane is modeled with deGennes' reptation model. The models are used to estimate flow fields necessary to carry out reactions and purification of genomic DNA on the membrane without shear degradation.

As an illustration of reactor performance, we demonstrate successful extraction of genomic DNA from *E. coli* cells, its purification, specific digestion with NotI restriction enzyme, and intercalation with POPO-1. The largest eluted DNA fragment was nearly 1 Mb-long.

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Using A Natural Material For Bacteria Concentration and Removal From Water

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In the last decade an extraordinary amount of research and development has focused on alleviating problems associated with contaminated water. With the majority of the World's population living on the brink of illness due to bacterial contamination in town water supplies, much of this attention has been focused on bacteria removal and sensors. Many current decontamination techniques are too technologically advanced for less developed countries, often resulting in their rejection by the societies they serve. Sensor work has also come across problems including poor sensitivity making it difficult to detect microorganisms at low concentrations. We have been testing a material extracted from the *Opuntia ficus-indica* cactus which could possibly address both of these problems in conjunction with one another. This material, referred to as cactus mucilage, has proven itself in the past as a viable flocculating agent for use in water contaminated with sediments and heavy metals. Flocculation tests, now focused on *Bacillus cereus* and *Escherichia coli*, have also given insight on the mucilage's ability to gather and concentrate bacterial contaminants from ion-rich water supplies. In columns with bacteria suspended in hard and soft water, flocculation begins immediately and is complete in approximately five to ten minutes with concentration rates of up to 99%. In addition to cleaning the water, the flocs formed with the mucilage could be removed from the water for sensor use. Cactus mucilage is an ideal material for water treatment and assessment because it is a naturally occurring, low cost material that is easy to obtain, process and use. Using this type of green chemistry, not only are bacteria concentrations significantly lowered in contaminated water, but also a highly concentrated volume of bacteria is produced that could potentially aid in biosensors.

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A Microfluidic Device For Concentrating High Molecular Weight DNA

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Direct Linear Analysis (DLA) technology obtains high content sequence information by optically mapping sequence specific fluorescent tags bound to elongated genomic DNA molecules in shear flows.[1] To facilitate sensitivity and throughput, we have implemented a high molecular weight DNA concentrating system by photopatterning a semi-permeable membrane inside the microfluidic device. This minimizes the fluid volume in which the DNA molecules reside prior to optical mapping leading to decreased read times. The membrane is selectively permeable to buffer ions but not high molecular weight DNA molecules allowing enhanced sample concentration at the membrane surface during electrokinetic transfer. In addition, the semi-permeable membrane allows elec-

trophoretic sample transfer into and throughout the microfluidic device avoiding hydrodynamic induced shear forces that can degrade the integrity of large DNA molecules. The device employs novel microfluidic channel geometries to limit the electric field strength to appropriate levels near the membrane surface to minimize both sample and membrane degradation while maintaining a sufficiently high electric field for rapid sample transfer. Additionally, ion polarization across the membrane due to selective membrane permeability is addressed by active buffer replenishment through devoted channels behind the membrane. This architecture is amenable to integration into electrophoretic systems requiring rapid sample concentration, positioning, and transfer between microfluidic components. This research was supported by the Department of Homeland Security Science and Directorate Technology.

[1] Chan et al., *Genome Research*, 2004, 14:1137.

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Single Microtubule Orientation on Patterned Non-fouling Surfaces

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Microtubule (MT) configuration and assembly are essential to cytoskeletal reorganization and vesicle transport. Through physical manipulation of microtubules we seek to investigate these sub-cellular processes and to fabricate lab-on-a-chip diagnostic tools. We have developed a straightforward method for on-demand orientation of single microtubules on lithographically patterned electrodes. A poly(ethylene glycol) self-assembled monolayer (SAM) passivates the electrodes to MT adsorption prior to inducing MT migration through application of an electrostatic potential. The nonfouling layer allows MTs to adsorb and orient on the patterned electrodes while preventing adsorption in the surrounding regions. In this way single microtubules can be coaligned to arbitrarily shaped submicron electrodes. This method has advantages over those which make use of kinesin, antibodies or biotin/streptavidin to bind microtubules as it is capable of on-demand adsorption and produces patterns of MTs without requiring subpatterns of these other biomolecules. In addition we present a facile method for producing the nonfouling SAM which prevents microtubule adsorption on silicon and gold surfaces, eliminating the need for casein, bovine serum albumin or other passivating treatments.

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Planar Lipid Bilayer Formation on a Laser-Drilled Quartz Substrate

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Quartz substrates are attractive platforms for ion channel research, owing to their improved dielectric properties over currently used substrates. Further, the piezoelectric properties of quartz make it an ideal candidate for probing mechanosensitive ion channels. Here we present evidence of planar lipid bilayer formation on a laser-drilled quartz substrate in transport measurements. Bilayer formation is evidenced by the incorporation of voltage-gated ion channels in the membrane.

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Exploring The Dynamic Actions Of Cellulolytic Enzymes In A Heterogeneous System With Micro-cantilever Technology

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The cellulolytic enzyme degradation process suffers from low efficiency and high cost because of the low activity of cellulases against their natural substrates cellulose, which is insoluble and crystalline in its native form. As a result, the degradation of crystalline cellulose becomes the rate-limiting step in the overall scheme of biomass conversion to ethanol. To address this problem, the development of a highly efficient and cost-effective cellulase has become one of the top priorities of the Advance Energy Initiative. Such an effort requires a thorough understanding of the mechanisms of cellulolytic enzyme actions including interactions between the cellulases and their native substrates. The current technologies such as ellipsometry and quartz crystal microbalance have not been able to provide with the level of sensitivity and resolution required for detailed characterization of the cellulolytic enzyme actions, especially the interaction between cellulase and glucan chains of cellulose, and the impact of such interaction on overall cellulose structure. To define cellulase actions in such a heterogeneous system, we focus on investigating the initial interaction between cellulase and its native substrate, crystalline cellulose by taking advantage of emerging micro-cantilever technology. We have constructed a micro-cantilever with a cellulose coating which allows us to detect the actions of cellulolytic enzymes in real time.